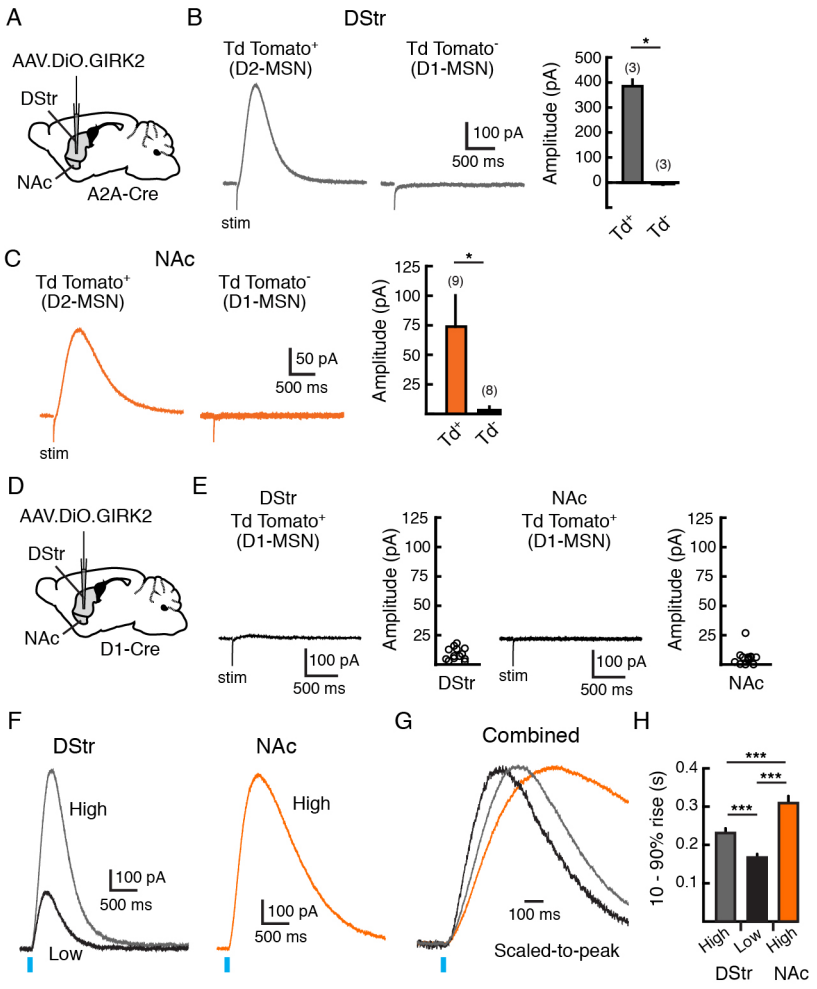
Supplementary Materials

Regional heterogeneity of D2-receptor signaling in the dorsal striatum and nucleus accumbens

Pamela F Marcott [1,2], Sheng Gong [1,2], Prashant Donthamsetti [3,4], Steven G. Grinnell [8,9], Melissa N. Nelson [8,9], Amy H Newman [5], Lutz Birnbaumer [6], Kirill A Martemyanov [7], Jonathan A Javitch [3,8,9], Christopher P Ford[1,2]

1 - Department of Pharmacology, University of Colorado School of Medicine, Anschutz Medical Campus, Aurora, CO 80045, USA
2 - Department of Physiology and Biophysics, Case Western Reserve University School of Medicine, Cleveland, OH 44106, USA
3 – Department of Pharmacology, Columbia University, New York, NY 10032, USA
4 – Current address: Department of Molecular and Cell Biology, University of California, Berkeley, California 94720, USA
5 – National Institute of Drug Abuse, NIH, Baltimore MD, 21224, USA
6 – Neurobiology Laboratory, National Institute of Environmental Health Sciences, North Carolina 27709, and Institute of Biomedical Research (BIOMED), Catholic University of Argentina, Buenos Aires C1107AAZ, Argentina
7 – Department of Neuroscience, The Scripps Research Institute, Jupiter, FL 33458, USA
8 – Department of Psychiatry, Columbia University, New York, NY 10032, USA
9 – Division of Molecular Therapeutics, New York State Psychiatric Institute, New York, NY 10032, USA



Supplemental Figure 1 (refers to Figure 1).

A) Injection schematic of AAV2/9.hSyn.DIO.tdTomato.GIRK2 into the DStr and NAc of A2A-Cre mice.

B, C) Representative traces and quantification showing the response to electrical stimulation in tdTomato$^+$ and tdTomato$^-$ cells of the DStr (B) and NAc (C). D2-IPSCs were evoked in all tdTomato$^+$ cells recorded.

D) Injection schematic of AAV2/9.hSynapsin.DIO.tdTomato.T2A.mGIRK2-1-A22A.WPRE into the DStr and NAc of D1-Cre mice.

E) Representative traces and quantification showing the response to electrical stimulation in tdTomato$^+$ cells of the DStr and NAc. D2-IPSCs were never seen in tdTomato$^+$ cells in either region.

F) D2-IPSCs recorded in the DStr with high and low intensity optogenetic stimulation

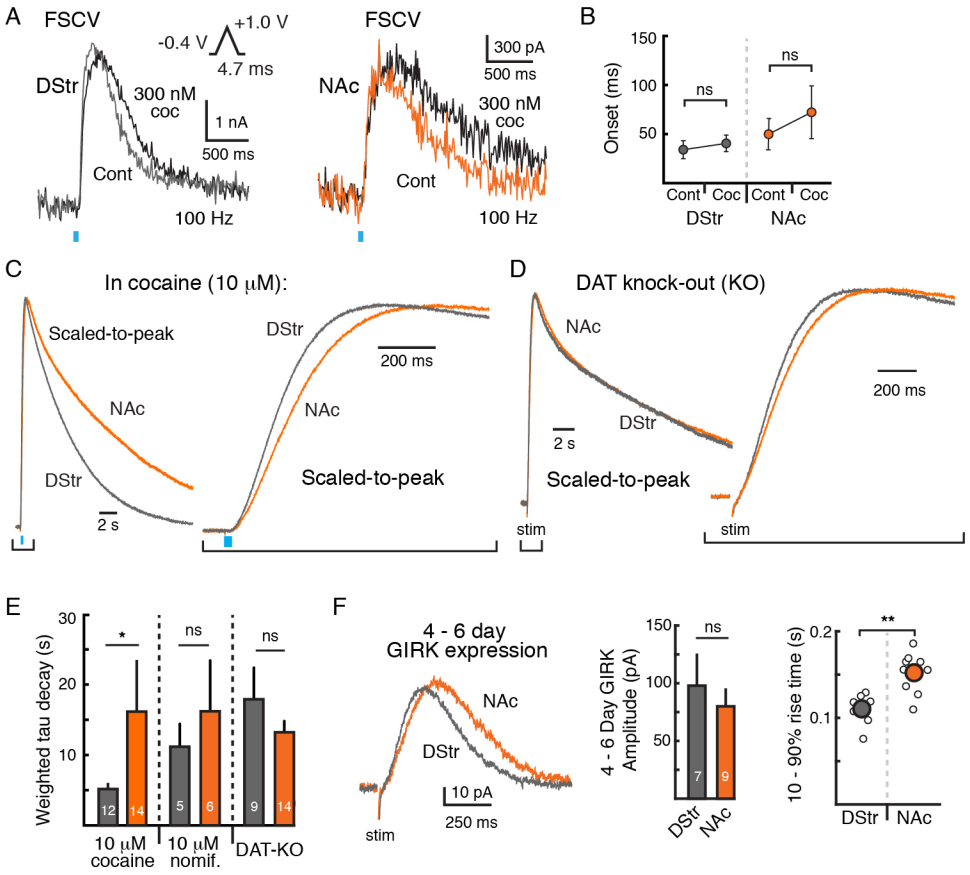
and NAc with high intensity stimulation.

G) IPSCs scaled to peak.

H) 10 – 90% rise time of D2-IPSCs.

Numbers in bar graphs represent number of cells recorded. Error bars indicate ± SEM.

ns, not significant = $p > 0.05$, * = $p < 0.05$, ** = $p < 0.01$.



Supplemental Figure 2 (refers to Figure 2).

A) Average optogenetically evoked FSCV traces (100 Hz) in the DStr and NAc under control conditions and in the presence of cocaine (300 nM, black). Inset: FSCV waveform.

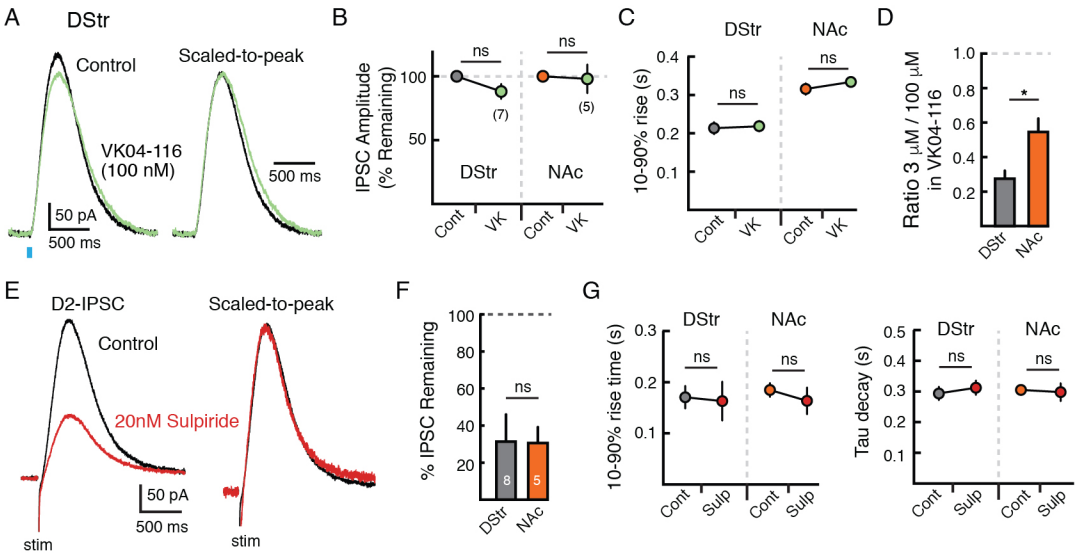
B) Quantification of the lag to onset (time to 2 s.d. above the noise) (DStr: p = 0.2, n = 5; NAc: p = 0.2, n = 5; Student's paired *t*-test).

C) Average traces of optogenetically evoked D2-IPSCs recorded in the presence of cocaine (10 μM) in the DStr (gray) and NAc (orange). Right: magnification of rise phase.

D) Average traces of electrically evoked D2-IPSCs recorded in the DStr (gray) and NAc (orange) of DAT-KO mice. Inset: magnification of rise phase.

E) Quantification of the weighted tau of decay of D2-IPSCs recorded in cocaine (10 μM), nomifensine (10 μM), and DAT-KO mice (Coc: $p < 0.05$, n = 12 – 14; Nomif: p = 0.6, n = 5 – 6; DAT-KO: p = 0.4, n = 9 – 14; Mann-Whitney test). The regional difference

in decay kinetics of the D2-IPSCs in the presence of cocaine (10 μM) can likely be attributed to incomplete inhibition of DATs.
F) Representative traces and quantification of IPSCs in DStr and NAc 4 – 6 days after AAV.GIRK2 injection (Amplitude: Mann-Whitney test, $p = 0.9$; 10 – 90% rise: DStr = 110 ± 7 ms, $n = 7$; NAc = 152 ± 8 ms, $n = 9$, $p < 0.01$, Mann-Whitney test).
Numbers in bar graphs represent number of cells recorded. Error bars indicate ± SEM. ns, not significant = $p \geq 0.05$, * = $p < 0.05$, ** = $p < 0.01$, *** = $p < 0.001$.



Supplemental Figure 3 (refers to Figure 6 and STAR Methods)

A) Representative traces of a D2-IPSC recorded in the DStr under control conditions and after bath application of the D3-receptor antagonist VK04-116 (100 nM). Right: scaled-to-peak.

B) Quantification of the percent of the IPSC remaining in VK04-116 (DStr: p = 0.1, n = 7; NAc: p = 0.9, n = 5; Student's paired *t*-test).

C) Quantification of the 10 – 90% rise time before and after addition of VK04-116 (DStr: p = 0.3, n = 7; NAc: p = 0.1, n = 5; Student's paired *t*-test).

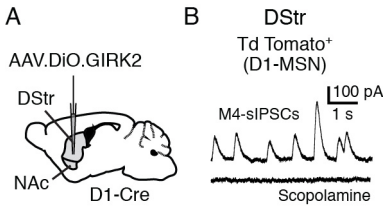
D) Quantification of the ratio of the outward current produced by 3 µM and 100 µM dopamine in the DStr and NAc (DStr = 28% ± 4%, NAc = 55% ± 8%; p < 0.05, Mann-Whitney test).

E) Representative traces of D2-IPSCs under control conditions (black) and after bath application of sulpiride (20 nM, red).

F) Quantification of the reduction in IPSC amplitude in both the DStr and NAc (p = 0.99, Mann-Whitney test).

G) Quantification of IPSC kinetics after addition of sulpiride (10 – 90% rise: DStr, p = 0.4; NAc, p = 0.2; tau decay: DStr, p = 0.2; NAc, p = 0.8; Student's paired *t*-test).

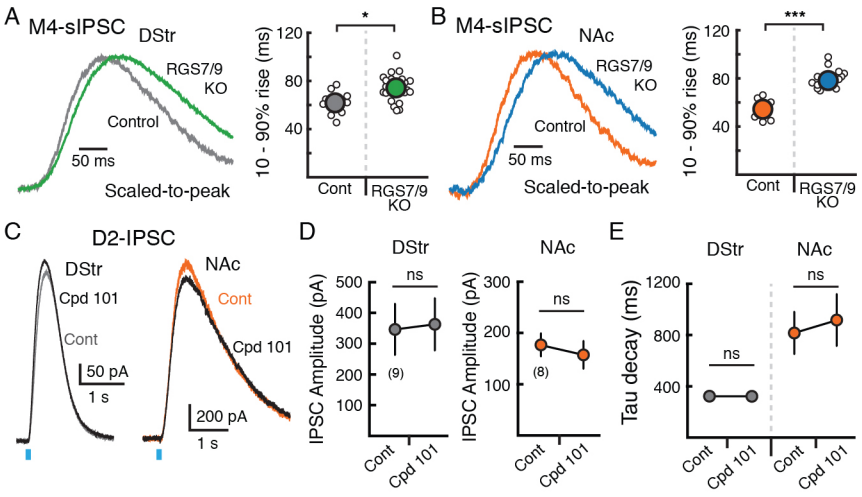
Numbers in bar graphs represent number of cells recorded. Error bars indicate ± SEM. ns, not significant = p ≥ 0.05, * = p < 0.05.



Supplemental Figure 4 (refers to Figure 6)

A) Schematic of the injection of AAV2/9.hSyn.DIO.tdTomato.GIRK2 into the DStr of D1-Cre transgenic mice.

B) Representative whole-cell recording showing spontaneous M4 muscarinic IPSCs (M4-sIPSCs) in the DStr of a tdTomato$^+$ MSN. M4-sIPSCs were present in all tdTomato$^+$ MSNs recorded (4/4). M4-sIPSCs were blocked by the muscarinic receptor antagonist scopolamine.



Supplemental Figure 5 (refers to Figure 7).

A) Average traces (overlaid and scaled-to-peak) of M4-sIPSCs in the DStr of RGS7/9 KO mice (green) and control littermates (gray). Right: Quantification of 10 – 90% rise time ($p < 0.05$, Mann-Whitney test).

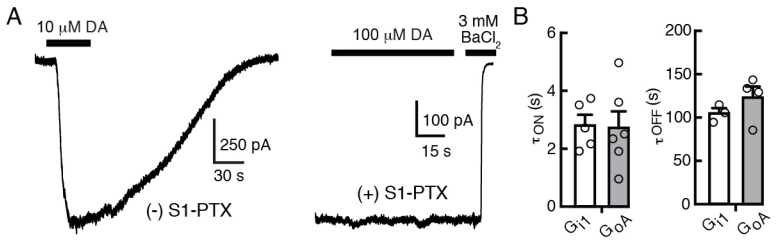
B) Average traces (overlaid and scaled-to-peak) of M4-sIPSCs in the NAc of RGS7/9 KO mice (blue) and control littermates (orange). Right: Quantification of 10 – 90% rise time ($p < 0.001$, Mann-Whitney test).

C) Representative whole-cell recordings of optogenetically evoked D2-IPSCs in the DStr (left, gray) and NAc (right, orange) before and after application of compound 101 (Cpd 101, black).

D) Quantification of the change in IPSC amplitude in Cpd 101.

E) Quantification of the change in tau of decay in Cpd 101.

Error bars indicate ± SEM. ns, not significant = $p \geq 0.05$, * = $p < 0.05$, *** = $p < 0.001$.



Supplemental Figure 6 (refers to Figure 8).

A) Left: Representative trace of a dopamine induced D2-receptor mediated GIRK current in HEK cells. Right: inactivation of endogenous $G\alpha_{i/o}$ with pertussis toxin (PTX) abolishes GIRK current.

B) There is no difference in the kinetics of dopamine induced D2-receptor mediated GIRK current in HEK cells.

	DStr		NAc	
D2R-GIRK	EC50 (μM)	95% CI (μM)	EC50 (μM)	95% CI (μM)
Control	5.9	4.0 to 9.0	1.3	1.0 to 1.9
Cocaine	8.8	5.5 to 14	6.1	4.5 to 8.4
RGS7/9 KO	6.7	3.9 to 11.7		
Go cKO	7.5	3.1 to 18.2	11.7	5.8 to 25.1
D2R axon collaterals	IC50 (μM)	95% CI (μM)	IC50 (μM)	95% CI (μM)
DA inhibition	4.8	3.3 to 7.1	2.1	1.5 to 2.9
OR-GIRK	EC50 (nM)	95% CI (nM)	EC50 (nM)	95% CI (nM)
Leu-enkephalin	320	180 to 550	340	160 to 700

Supplemental Table 1. *Summary of concentration-response curve data from MSNs. Related to Figures 4, 5, 6, 7 and 8.*